Filed on behalf of: Party University of Iowa Research Foundation, et al.

Paper No. ___

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Moore)

UNIVERSITY OF IOWA RESEARCH FOUNDATION,
COLEY PHARMACEUTICAL GROUP, INC., and
THE UNITED STATES OF AMERICA, as represented by the Department of Health and Human Services
(6,207,646 B1)
Junior Party,

v.

THE REGENTS OF
THE UNIVERSITY OF CALIFORNIA
(09/265,191),
Senior Party.

Patent Interference No. 105,171

DECLARATION OF CY STEIN
IN OPPOSITION TO CALIFORNIA'S PRELIMINARY MOTIONS

Iowa EXHIBIT 2060
Iowa v. California
Interference 105,171

I, Cy Stein, declare that:

1. I make this declaration in support of Iowa's Oppositions to California's Preliminary Motions in the above-identified proceeding.

2. I am presently a Professor of Medicine, Urology and Molecular Pharmacology and Head of Medical Genitourinary Oncology at Albert Einstein College of Medicine, Bronx, NY. I am also an Attending Physician at Montefiore Medical Center, Bronx, NY. Prior to holding these positions, I held the following positions:

   - Associate Professor of Pharmacology and Medicine, Columbia University, College of Physicians and Surgeons (1996-2003);
   - Assistant Professor of Medicine and Pharmacology, Columbia University, College of Physicians and Surgeons (1992-1995);
   - Assistant Professor of Medicine, Columbia University, College of Physicians and Surgeons (1990-1995);
   - Senior Staff Fellow, National Cancer Institute, Bethesda, Maryland (1988-1990);
   - Clinical associate, National Cancer Institute Bethesda, Maryland (1985-1988);
   - Resident, Internal Medicine, The New York Hospital-Cornell Medical Center, New York, New York (1983-1985); and
   - Intern, Internal Medicine, The New-York Hospital-Cornell Medical Center, New York, New York (1982-1983).

3. I have a B.A. from Brown University (1974), a Ph.D. in Organic Chemistry from Stanford University (1978), and an M.D. from Albert Einstein College of Medicine (AECOM) (1982).

4. My Editorial and Advisory Positions include:

Co-Editor-in-Chief: Oligonucleotides (formerly Antisense and Nucleic Acid Drug Development and Antisense Research and Development; Mary-Ann Liebert Publishers), and

Series Editor: Perspectives in Antisense Science (Kluwer Academic Publishers; four volumes published to date).

Editorial Advisory Boards:

- Cancer Gene Therapy (Stockton Press);
- Molecular Cancer Therapeutics (American Association for Cancer Research);
- Clinical Cancer Research (American Association for Cancer Research);
- Oncology Research (Cognizant);
- BioDrugs (Adis International); and
- Clinical Prostate Cancer.

5. I am a named author on over 100 published articles, one of which is California Exhibit 1009. I am also an author on over 60 book chapters, reviews and editorials. I am an inventor on 7 United States Patents and 4 pending United States Patent Applications. Many of these patents and applications relate to phosphorothioate oligonucleotides and antisense technology. A copy of my Curriculum Vitae is attached as Iowa Exhibit 2061.

6. In preparing this declaration, I reviewed:

- U.S. Patent 6,207,646 to Krieg et al. (CA Exhibit 1001) (hereinafter "'646' patent");
- Sato et al. (1996) Immunostimulatory DNA sequences necessary for effective intradermal gene immunization, *Science*, 273:352-354 (CA Exhibit 1002) (hereinafter "Sato");
- Kataoka et al. (1992) Antitumor activity of synthetic oligonucleotides with sequences from cDNA encoding proteins of *Mycobacterium bovis* BCG. *Jpn. J. Cancer Res.* 83:244-247 (CA Exhibit 1005) (hereinafter "Kataoka");
- Agrawal, S., et al. (1991) Pharmacokinetics, biodistribution, and stability of oligodeoxynucleotide phosphorothioates in mice. *Proc. Natl. Acad. Sci.* USA 88:7595-7599 (CA Exhibit 1008) (hereinafter "Agrawal");
- Stein and Cheng (1993) Antisense Oligonucleotides as Therapeutic Agents – Is the Bullet Really Magical? *Science*, 261:1004-1012 (CA Exhibit 1009) (hereinafter "my Science article"); and
- Declaration of Dr. Marsha Wills-Karp (CA Exhibit 1011).

7. I have studied oligonucleotide backbone modification for more than 17 years. I have continued to study phosphate modified oligonucleotides to this day. I consider myself to be at least of ordinary skill in the field of immunostimulatory nucleic acids and to have more than ordinary skill in the particular field of phosphate modified nucleic acids.

8. My 1993 *Science* paper is a review of phosphate modified oligonucleotides in the antisense field.

9. All of my statements about the knowledge and expectations of one of skill in the art with respect to backbone modifications and the above articles, Sato, Kataoka, Agrawal and my article, apply equally to 1994, 1995 and 1996 unless otherwise noted.

10. Those of ordinary skill in the art did not know the mechanisms through which the nucleic acids of Katoaka or Sato achieved immune stimulation.

11. Without knowledge of the mechanisms through which these nucleic acids achieved immune stimulation, it would have been completely unpredictable to one of ordinary skill in the art whether a phosphate backbone modification would totally destroy the immunostimulatory capability of the Kataoka or Sato nucleic acids.

12. Phosphate backbone modifications were, and still are, known to have unpredictable and undesirable effects on nucleic acids. For example, phosphate backbone modifications may affect the base pairing of nucleic acids, such that double-stranded molecules "unzip," (i.e., have weakened or absent bonds between the complimentary base pairs of the two strands) and/or single-stranded molecules do not bond properly to complimentary RNA or DNA strands. In addition, some phosphate modifications could affect solubility, melting point, and even other characteristics of nucleic acids. These effects differ among the type, number and location of the modifications.

13. The specific phosphate backbone modifications, phosphorodithioate and phosphorothioate, involve replacing the usual one or more oxygen atoms that are associated with the bond between base pairs in a nucleic acid strand with the much larger sulfur atom.

14. Among the complications introduced by phosphorothioate modification is the creation of stereochemistry. The sulfur in a phosphorothioate modification introduces stereochemistry at each bond where it is present, creating distinct versions of the molecule.

15. The two stereochemical forms of the phosphorothioate linkage each produce molecules with biological activities that can be distinct from each other, and distinct from an unmodified nucleic acid, having the same base pairs.

16. Because stereochemistry is introduced at each site with a phosphorothioate bond, a molecule with several or many such bonds is actually an enormously complex mixture of different chemical entities with unpredictable properties.

17. This stereochemistry of phosphorothioates was known well prior to 1994 and continued to be known to those of ordinary skill in the art through 1996, to the present. One of skill in the art would not have known whether the introduction of stereochemistry would affect immunostimulation. This stereochemistry does not occur with the usual oxygen.

18. In addition to the stereochemistry, the sulfur atom can have further effects on the activity of the nucleic acid simply due to its being much larger than the oxygen.

19. Phosphorodithioate modifications, although not known to uniformly or regularly introduce stereochemistry, still are known to have unpredictable effects. They may distort the shape of the molecule so as to alter its ability to bind to targets it usually binds to.

20. My 1993 *Science* paper shows that phosphorothioate modifications can have unpredictable effects on an oligonucleotide. In fact, phosphorothioate can unpredictably redirect oligonucleotide activity to create biological activity against targets where there previously was none.

21. Phosphorothioate modifications have many more biological effects than simply reducing oligonucleotide degradation *in vivo*. As detailed in my 1993 *Science* paper those effects were not well understood.

22. For example, at p. 1098, col. 3 and p. 1009, cols. 1 and 2, we detail four possible explanations for the non-specific antisense effects of a particular phosphorothioate antisense oligonucleotide.

23. Many of the non-specific effects of a first phosphorothioate antisense molecule are not the same as, and cannot be predicted from, the non-specific effects of a second phosphorothioate antisense molecule having a different nucleotide sequence.

24. One of ordinary skill in the art would have known that shape could have an impact on immunostimulatory activity. In the absence of Dr. Krieg's work it would not have been known from 1994 through 1996 whether a phosphorothioate bond or phosphorodithioate bond would substantially change the shape of the oligonucleotide so as to totally destroy immunostimulatory ability.

25. One of ordinary skill in the art would have known that binding targets and binding capabilities could impact immunostimulatory capabilities of a nucleic acid. In the absence of Dr. Krieg's work, it would not have been known from 1994 through 1996 whether a phosphorothioate bond or phosphorodithioate would substantially change or redirect the binding of an oligonucleotide necessary for immunostimulation.

26. The characteristics of the 5 ' and 3 ' termini would have been understood by one of skill to have a potential impact on immunostimulatory activity. It was not known from 1994 through October 30, 1996 whether an unmodified 5 ' or 3 ' end was necessary for immunostimulatory activity.

27. In Kataoka, there were palindromes that were inactive, therefore, even though Kataoka attributed the "activity" of the oligonucleotides to palindromes, it would have been unclear to

one of ordinary skill in the art what characteristics of the molecule were actually critical for activity.

28. In the absence of Dr. Krieg's work as shown in the '646 patent and the 1995 *Nature* paper, from 1994 through 1996, it would not have been predictable that a phosphate backbone modification to a molecule shown to be "active" in Kataoka would allow the molecule to retain its immune stimulatory effects.

29. The Sato reference is about the insertion of immune stimulatory sequences into plasmids.

30. My paper has nothing to do with plasmids or phosphate backbone modifications to plasmids.

31. Agrawal also has nothing to do with plasmids or phosphate backbone modifications to plasmids.

32. One of ordinary skill in the art would not extend the teachings of my paper or the Agrawal reference to plasmids. My paper and the Agrawal reference deal with relatively short chemical structures, whereas, plasmids are complex biological systems, intended to carry out functions such as replication of themselves and transcription of mRNA for protein production.

33. To my knowledge therapeutic plasmids were not known to contain phosphate backbone modifications generally, or phosphorothioate and phosphorodithioate backbone modifications, specifically.

34. A phosphate backbone modification would be expected by one of ordinary skill in the art to impair if not destroy the functionality of a plasmid, including the replication and transcription functions.

35. Even if one were to obtain a functional phosphorothioate modified plasmid, the concerns with respect immunostimulation discussed above would still exist.

36. For the above reasons, those of ordinary skill in the art would not have used a phosphate backbone modification in relation to a plasmid based on my paper or the Agrawal reference.

37. Sato did describe an *in vitro* experiment with oligonucleotides, however, as noted with respect to the Kataoka oligonucleotides, since the mechanism through which the nucleotides stimulated the immune system was not known, one of ordinary skill in the art would have considered the results of phosphate backbone modifications to the oligonucleotides discussed in Sato to be very unpredictable. Such persons would not have had reason to believe that modifying those oligonucleotides with phosphorothioate would still allow them to retain the immune stimulating activity for the same reasons discussed above with respect to the Kataoka oligonucleotides.

38. With the exception of Dr. Krieg's work, there was no teaching in the art prior to October 1996, which disclosed results indicating that phosphate modified nucleic acids could retain the same immunostimulatory properties as unmodified nucleic acids.

39. In addition, one of ordinary skill in the art would not even understand Sato as suggesting oligonucleotides for a therapeutic purpose.

40. I have also reviewed claims 14, 18 and 22 of the '646 patent. None of these claims recite a phosphate modified backbone. In view of the knowledge and skill in the art prior to April 1995, one of ordinary skill in the art could not predict whether a phosphorothioate or phosphorodithioate modification in a CpG mediated immunostimulatory nucleic acid would alter or totally destroy the immunostimulatory properties of the nucleic acid. Even after April 1995, any expectation that the nucleic acids would not be adversely affected would only be based upon the teachings of Dr. Krieg in his April, 1995 *Nature* article or his patent disclosures.

41. Similarly, I understand that claim 31 recites a phosphate backbone modification. In view of the knowledge and skill in the art prior to Dr. Krieg's April 1995 *Nature* paper one of ordinary skill in the art could not predict whether a phosphate modified backbone would alter or totally destroy the immunostimulatory properties of the nucleic acid.

42. As discussed above, phosphate backbone modifications were known, and continue to be known, to be highly unpredictable and to have potentially adverse affects on the activity and structure of nucleic acids.

I, Cy Stein, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this document, the '646 patent and any patent which may issue that is a continuation or continuation-in-part of the '646 patent.

Date: 8/31/04

Cy Stein